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(54) Material for removing HIV and its related substances.

(57) A material for removing human immunodeficiency virus (HIV) and its related substances from blood, plasma, or other body fluids is provided. The material comprises a porous substrate on which sulfuric group substantially in the form of a salt is immobilized. A convenient removal of HIV and its related substances at a high efficiency is enabled by the use of the material.

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FIELD OF THE INVENTION

This invention relates to a material for removing human immunodeficiency virus (HIV) and the related substances from a body fluid. Mor illustratively, this invention relates to a membrane which is capabl of selectively adsorbing the HIV and its related substances to enable removal of such substances from protein rich solutions such as blood, plasma, and solutions containing blood components.

BACKGROUND OF THE INVENTION

Many virus particles have an isoelectric point (pI) of from 3 to 6, and such viruses are negatively charged in neutral pH range. When such viruses are located in highly pure water, the viruses may be adsorbed onto an appropriate substrate by electrostatic interaction between the virus and the substrate.

Japanese Patent Application Laid-Open No. 3(1991)-123630 discloses a virus removing material comprising a substrate such as a porous membrane having polyvinylpyridinium structure thereon. This porous material, however, suffered from non-specific adsorption of proteins, and when the material was used for virus removal from fluids such as blood and plasma, which usually contain proteins at a high concentration, it failed to selectively adsorb the virus.

Another filter for removing virus from body fluids and other protein solutions is disclosed in Japanese Patent Application Laid-Open No. 2(1990)-167232. This filter comprises a regenerated cellulose membrane having a pore size smaller than the HIV particle, and the passage of the HIV particles upon filtration through the membrane is thereby inhibited. Such small pore size, however, also resulted in low filtration rate as well as frequent clogging. In addition, there has been reported that the pathogenicity of the HIV is found not only in the HIV particle, but also in the envelope proteins such as gp120 and gp160 (see, for example, *Microbiological Reviews*, Mar. 1993, pages 183-289, "Pathogenesis of HIV infection".) The pore size of this filter is not small enough to capture the gp120.

Another virus removing material is disclosed in WO89/01813, that utilizes biological affinity for the removal of the virus from the blood or plasma. This material comprises a substrate having immobilized thereon a virus-binding site (receptor) of a cell, that is found on the surface of the cell. Production of such material, however, involves complicated steps of isolation of the receptor from the cell and its purification followed by immobilization of the purified receptor onto the substrate. Use of such receptor of biological origin also resulted in an increased cost and some risk of unstable performance and alteration in the quality after storage.

Japanese Patent Application 2(1990)-36878 (USP 5,041,079) discloses a material for removing HIV and its related substances comprising a solid substance with a weakly acidic or weakly alkaline surface at a pH of from 2.5 to 6.9 or from 7.4 to 10.5. This material may typically have -COOH or -SO_3H on its surface, and there is clearly stated that "substances whose surface pH is in the range 7.0 to 7.3 (e.g. nonpolar polypropylene, quartz, and cation-exchange resins of -SO_3Na type) are unable to absorb HIV and/or its related compounds" (col. 2, lines 29 to 33 in USP 5,041,079). Furthermore, it has been confirmed that the body fluid would undergo an alteration in its pH upon contact with such an HIV removing material, and use of such material is not necessarily favorable for blood and other body fluids since such alteration in the pH may result in the denaturing of the proteins.

SUMMARY OF THE INVENTION

In view of the situation as described above, an object of the present invention is to provide a material by which HIV and its related substances can be removed from protein rich solutions such as blood and plasma preparations at a high selectivity and by a convenient operation.

To attain such object, there is provided by the present invention a material for removing HIV and its related substances comprising a porous substrate having a plurality of pores wherein said pores have sulfuric group substantially in the form of a salt such as potassium or sodium salt on their surface.

The porous substrate is preferably a porous membrane comprising a hydrophobic polymer such as polypropylene, polyethylene or polyvinylidene fluoride having an average pore diameter of from 0.1 to 1 µm ; and the porous membrane may preferably have a polysulfuric compound loaded on its pore surface.

The polysulfuric compound may be loaded on the porous membrane via an intervening hydrophilic graft chain having a l w protein adsorptivity; such as those comprising an acrylamide polym r, a polyether polymer, or a polyalkoxyalkylacrylate polymer such as polymethoxyethylacrylate.

Preferably, the intervening hydrophilic graft chain is a side chain grafted on the porous substrate, which comprises alkoxyalkyl acrylate and/or glycidyl (meth)acrylate. Alkyl and alkoxy are understood in the present application as meaning (C_1-C_4)alkyl and (C_1-C_4)alkoxy, respectively.

"Low protein ads rptivity" means that the affinity (adsorption) betw en the intervening hydrophilic graft chain and various prot ins in blood is low.

The polysulfuric compound may preferably be selected from d xtran sulfate ; cellulose sulfate ; curdlan sulfat ; polymers and copolymers of sulfoethylacrylat , vinyl sulfat , and styrene sulfonic acid.

The material may preferably have 1 x 10^{-4} mol/g or more of the sulfuric group on the porous membrane substrate, preferably the sulfuric group is a salt of potassium or sodium.

According to the present invention there is also provided a method for removing HIV and its related substances from blood by adsorption simultaneously with the separation of the plasma from the blood. In this method, the blood is filtered through a porous membrane having an average pore diameter of from 0.1 to 1 μm wherein the pores have sulfuric group substantially in the form of a salt loaded on their surface.

According to the present invention there is also provided a method for removing HIV and related substances from blood or plasma by adsorption. In this method, the blood or plasma is contacted with a porous substrate wherein the pores have sulfuric group substantially in the form of a salt loaded on their surface.

DESCRIPTION OF THE INVENTION

In the present invention, the "material for removing human immunodeficiency virus (HIV) and its related substances" is a material that removes or inactivates the HIV and related substances, and preferably, a material that removes the HIV and its related substances from blood or other body fluids by adsorption onto the material.

The "HIV related substances" include glycoproteins constituting the HIV such as gp120 and gp160, that are reported to be pathogenic, and complexes of such glycoproteins with a biological component.

The material for removing HIV and its related substances has on its surface sulfuric group substantially in the form of a salt, namely, in the form of potassium salt ($-SO_3K$), sodium salt ($-SO_3Na$), or the like in contrast to the sulfuric group of proton type ($-SO_3H$) as employed in Japanese Patent Application Laid-Open No.2(1990)-36878 (USP 5,041,079). As mentioned above, when a protein solution such as a body fluid is brought in contact with the material having the sulfuric group of proton type on its surface, the protein that became in contact with the sulfuric group would undergo an alteration in its pH by ion exchange reaction, and the HIV inactivation thus proceeds simultaneously with the adsorption and denaturing of other proteins. In contrast to such material, the material wherein the sulfuric group is in the form of a salt exhibits reduced non-specific protein adsorption as well as improved removal of the HIV and related substances including the gp120.

The material for removing HIV and its related substances may preferably have 1 x 10^{-4} mol/g or more of the sulfuric group on its surface although the amount of the sulfuric group required may vary in accordance with the configuration of the material. When the amount of the sulfuric group is less than 1 x 10^{-4} mol/g, the material would exhibit an insufficient removal of the HIV and gp120. The material may more preferably have 2 x 10^{-4} mol/g or more of the sulfuric group preferably localized on its surface that becomes in contact with the liquid to be treated.

The characteristic feature of the material for removing HIV and its related substances of the present invention is its ability to selectively remove the HIV and its related substances from the liquid such as blood that contains a variety of proteins, and it is such feature that enables the material of the present invention to be used in treating the patient suffering from HIV infection such as AIDS (acquired immune deficiency syndrome) and ARC (AIDS related conditions). In the treatment of AIDS, it is important to prevent the virus from spreading, to reduce the load imposed by the virus, and to inhibit the virus replication. Removal of HIV, gp120 and the like from the body fluid of the patient suffering from HIV blood conditions should result in the reduction of the virus load and prevention of the virus spread, and hence, in the improvement of the QOL (quality of life) of the patient and suppression of the disease progress.

The removal of the HIV and its related substances by the material of the present invention is represented by the reduction in the HIV infectious titer calculated in terms of median tissue culture infectious dose ($TCID_{50}$)/ml by the use of the material. For example, when a disk-shaped filter having a diameter of 25 mm is used by passing 2 ml of plasma having an infectious dose of from several tens to several hundreds $TCID_{50}$/ml (corresponding to the treatment of about 10 liters of plasma or about 20 liters or more of blood by a module having a membrane area of 0.5 m^2), the filter should exhibit an HIV removal of 85% or higher, and preferably, 90% or higher, and more preferably, 95% or higher.

In the present invention, loading of the sulfuric group on the material means chemical binding or immobilization f the sulfuric group on the material surface in a manner that the sulfuric group would n t dissolve into water or blood. Such loading may b accomplished by various means, for example, by introducing a functional radical such as epoxy, amino, aldehyd , carb xyl, hydroxyl, r acid chloride group on th surface of th polymer material by such means as graft polymerization, coating, chemical modification, oxidizati n, and cross linking, and immobilizing the polysulfuric compound directly on the thus treated material by using or without using a

coupling ag nt, or indirectly with an intervening spacer.

In a preferred method, a surface graft chain having a reactive functional radical may b introduced n the surface of the polymer material, and a polysulfuric compound may be immobilized on the reactive functional radical. In another method, the material surface is sulfat d with sulfuric acid or sulfite to form a surface structure wherein a polysulfuric compound is loaded on the material surface. Preferably, such sulfation of the material surface is carried out after graft polymerizing a compound having epoxy group or hydroxyl group on the material surface.

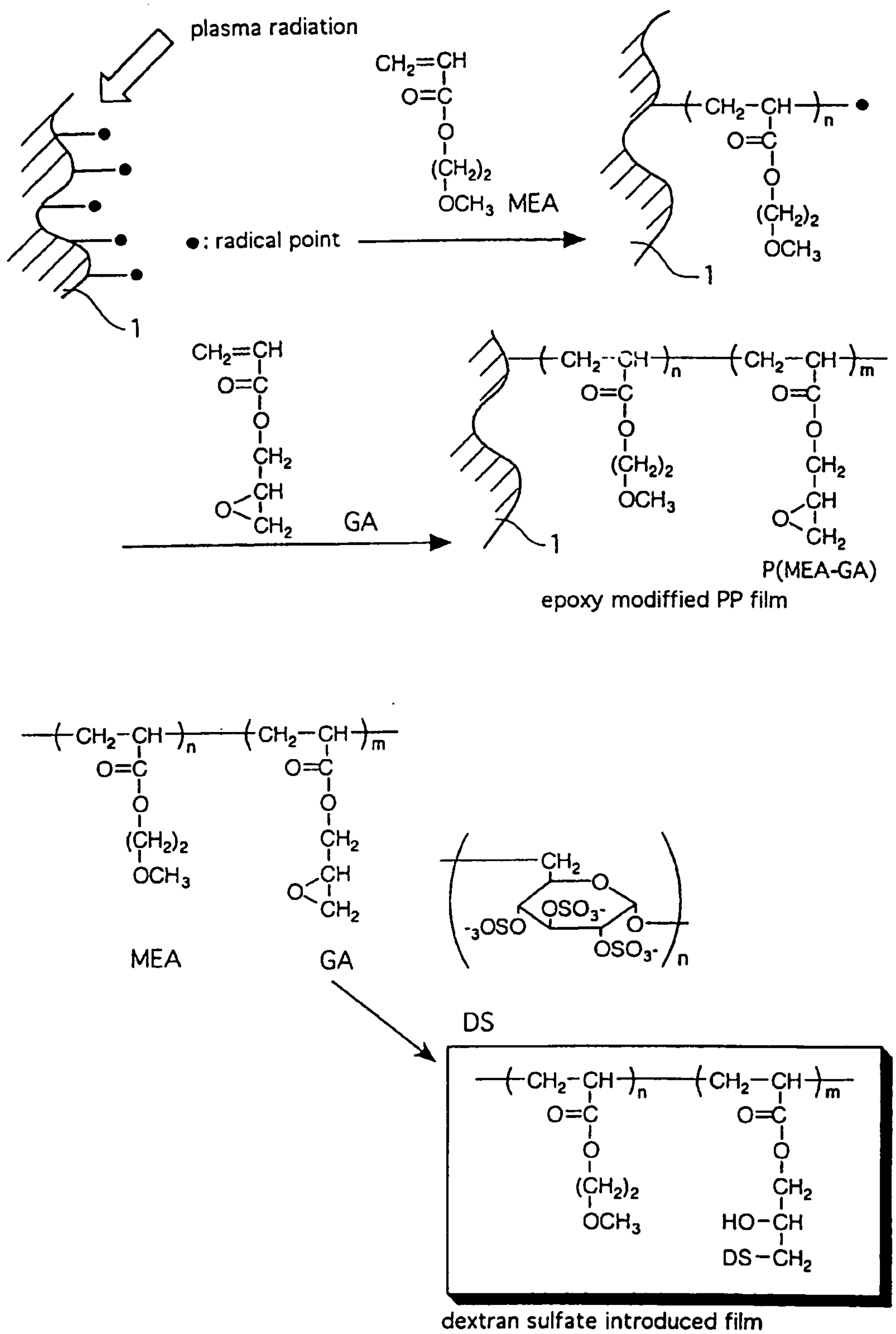
The graft chain having a reactive functional radical may preferably comprise a monomer component such as glycidyl acrylate and glycidyl methacrylate having epoxy group therein, since the epoxy group may be changed to an amino group for reacting with a sulfuric group.

The polysulfuric compound is not limited to any particular type so long as it has a plurality of sulfuric groups in its molecule. Exemplary polysulfuric compounds are sulfated polysaccharides such as dextran sulfate, cellulose sulfate, and curdlan sulfate ; and polymers and copolymers of a sulfonated monomer such as sulfoethylacrylate, vinyl sulfate, and styrene sulfonic acid. Preferably, the polysulfuric compound is immobilized on the material by forming a hydrophilic graft chain exhibiting a low protein adsorptivity which comprises a polymer having a glass transition temperature (Tg) of up to 300K such as an acrylamide polymer, a polyether polymer, a polyalkoxyalkylacrylate polymer, glycidyl (meth)acrylate or the like on the material surface ; introducing the above-described surface graft chain having a reactive functional radical ; and immobilizing the polysulfuric compound on the reactive functional radical.

An example of the methods for producing the material of this invention is shown schematically hereinafter.

The polyolefin membrane 1 such as polypropylene is irradiated with plasma to introduce radical points on the surface, and the surface is contacted with an alkoxy alkyl acrylate gas and/or a glycidyl (meth)acrylate gas to introduce graft polymerized side chains having active groups such as epoxy groups or hydroxyl groups.

Optionally, the active groups may be converted in other active groups, which may then be reacted with sulfuric groups, preferably in an aqueous solution.



The material for removing HIV and its related substances of the present invention is not limited in its con-

figuration and may have any desired configuration such as a fiber, a filter, r a tube. Preferably, the material is a filter having an av rage pore diameter of from 0.1 to 50 μm comprising a flat membrane, a holl w fiber, a woven fabric, or a nonwoven fabric.

When the material of the inv ntion is used for filtering plasma or a culture medium, the filter may preferably have an average pore diameter of 0.1 to 10 μm. The pore diameter of as small as less than 0.1 μm will result in an insufficient filtration rate. The average pore diameter as used herein is the value determined by Perm-Porometer (manufactured by Porous Materials, Inc.) described in ASTM-F316, and this value satisfactorily reflects the actual pore diameter.

Preferably, the filter may also have a water transmission of at least 10 ml/min/m^2/mmHg, and more preferably, at least 100 ml/min/m^2/mmHg when measured at a pressure of 0.7 kg/cm^2 and at 25°C ± 2°C, and then, the filter can be used at a relatively low filtration pressure.

When the material of the invention comprises a nonwoven fabric substrate, the nonwoven fabric substrate may comprise either a monofilament or a multifilament, but the filament should have an average diameter (an average of major diameter and minor diameter determined by observation with scanning electron microscope) of up to 100 μm and preferably, up to 50 μm. The membrane filter will then have a sufficient surface area, and hence, a sufficient adsorption site. It is also possible to use a profile filament, or alternatively, a porous filament. The filter material of the present invention may have a porosity (percentage of the pore volume) as determined by the following formula (A) of at least 20%, and preferably, at least 50%.

$$\text{Porosity (\%)} = \frac{\text{Volume of the pores}}{\text{Volume of (the pores and the filter material)}} \times 100$$

The material for removing HIV and its related substances of the present invention may comprise any desired material including natural polymers such as cellulose and its derivatives; and synthetic polymers such as polyolefin, polyamide, polyimide, polyurethane, polyester, polysulfone, and polyacrylonitrile. Preferably, the material of the invention may comprise a material with a high dimensional stability that may experience little swelling upon contact with water. Exemplary such materials include hydrophobic polymers such as polypropylene, polyethylene and polyvinylidene fluoride. The material used in the present invention may preferably have its surface modified, polymerized or treated by hydrophylic materials to introduce sulfuric group and to prevent it from adsorption of proteins.

As described above, the solution treated by the material of the present invention may include various proteins in addition to the HIV and its related substance. Exemplary solutions that may be treated by the material of the invention include body fluids such as blood, plasma, serum and urine and a liquid containing such a body fluid; and tissue or cell culture medium, supernatant thereof, and a liquid containing such a culture medium or supernatant.

The blood or blood containing solution treated by the material of the present invention may preferably be treated while removing blood cells.

The solution treated that may possibly contain HIV or its related substance is contacted with the material of the present invention to thereby reduce the HIV activity or the risk of HIV infection. The material may be contacted with the solution by batchwise immersion of the material in the solution; or by continuous passage of the solution through the material in the form of a column or a filter, the filtration through the filter-shaped material being the most preferred.

When the material for removing the HIV and its related substance may be in the form of a porous filter or a bead. Preferably the porous filter has an average pore diameter of from 0.1 to 1.0 μm, and has the sulfate group on its pore surface substantially in the form of a salt, it may be used as a horizontal filter in the filtration of blood to thereby accomplish plasma separation simultaneously with the removal by adsorption of the HIV and its related substances. Use of such filter also enables a convenient preparation of the HIV removing system.

The material and the method for removing HIV and its related substances of the present invention is further described by referring the Examples of the invention and Comparative Examples, which by no means limit the scope of the invention.

EXAMPLES

Examples 1 and 2 and Comparative Examples 1 and 2

A polypropylene (pp) membran having an average pore diameter of 0.5 μm, a porosity of 58%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-methoxyethyl acrylate (MEA) gas (1.0 Torr) for 3 minutes, and glycidyl acrylate (GA) gas (0.7 Torr) for 7 minutes to promote surface graft polymerization. The resulting hydrophilic porous membrane having ep xy

group introduced on its surface was immersed in aqueous ammonia of high concentrati n at 40°C for 48 hours to convert th epoxy group to amino group and produce an aminated hydrophilic porous membrane.

In the meanwhil , dextran sulfate (DS) (manufactured by Sigma; mol cular weight, 5,000; degree of sulfation, 2.2) was xidized with sodium periodate at a predetermined concentration (5% by weight) t produce aldehydated dextran sulfate.

The aminated hydrophilic porous membrane was reacted with the aldehydated dextran sulfate in water, and the resulting membrane was reduced with a 10 % by weight aqueous solution of $NaBH_4$ to strengthen the bond between the sulfate group and the membrane.

Sample membranes of Examples i and 2 wherein the sulfate group on the porous membrane is in the form of Na salt were prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

Sample membranes of Comparative Examples 1 and 2 wherein the sulfate group on the porous membrane is in the form of $-SO_3H$ were prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

The amount of the sulfate group introduced on the membrane was determined by acid base titration (NaOH-HCl back titration).

The sample membrane was set on a Swinrock Filter Holder (manufactured by Nuclepore; diameter, 25 mm), and 5 ml of HIV-containing human plasma (HIV infectious titer: 174 $TCID_{50}$/ml) was filtered through the membrane to measure HIV infectious titer and content of p24 before and after the filtration to thereby calculate the removal of the HIV and p24. The results are shown in Table 1. Removal of gp120, which is the glycoprotein found in the HIV envelope, was measured by filtering 5 ml of 2% FCS-containing RPMI 1640 medium containing HIV-containing human plasma through the membrane to measure the concentration of gp120 before and after the filtration, from which the gp120 removal was calculated. The results are also shown in Table 1.

The HIV-containing human plasma was prepared by cultivating HIV (HTLV-IIIB) persistently infected cell, Molt-4/HIV in 10% FCS-containing RPMI1640 and collecting the HIV solution by centrifugation, and adding human plasma to the HIV solution.

The HIV infectious titer was determined in terms of median tissue culture infectious dose ($TCID_{50}$)/ml by observing the cell morphology with naked eye and fluorescent antibody technique.

The p24 antigen was quantitated with RETRO-TEK p24 Antigen ELSA manufactured by Cellular Products, Inc.

Table 1

	Sulfate group		% removal		
	Amount, mol/g	Type	HIV	gp120	p24
Example 1	7.2×10^{-4}	$-SO_3Na$	99.2	≧ 95	≧ 98
Example 2	3.7×10^{-4}	$-SO_3Na$	97.2	≧ 95	≧ 98
Comparable Example 1	7.2×10^{-4}	$-SO_3H$	54.4	62	58
Comparable Example 2	3.7×10^{-4}	$-SO_3H$	58.2	55	52

Example 3 and Comparative Example 3

A polyvinylidene fluoride membrane having an average pore diameter of 0.4 μm, a porosity of 64%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with glycidyl acrylate gas (0.7 Torr) for 3 minutes to promote surface graft polymerization and produce a porous membrane having epoxy group introduced on its surface.

In the meanwhile, amino group was introduced into dextran sulfate (manufactured by Sigma; molecular weight, 5,000; degree of sulfation, 2.2) by means of 4-aminobutyldiethoxymethylsilane, and the aminated dextran sulfate was immobilized on th above-described porous membrane having epoxy group introduced thereon.

Sample membrane of Exampl 3 wherein the sulfate group is in the f rm of Na salt was prepar d by neutralizing the membrane with sodium hydroxide and thor ughly rinsing the membrane with distilled water.

Sample membrane of Comparative Example 3 wherein the sulfate group is in the form f $-SO_3H$ were pre-

par d by treating the membrane with 0.1N dilute hydrochloric acid and rinsing th membrane with distilled water.

The membranes w r evaluated for their performanc of HIV removal by repeating the proc dure of Example 1.

The results are shown in Table 2.

Table 2

	Sulfate group		% removal of
	Amount, mol/g	Type	HIV
Example 3	5.3×10^{-4}	$-SO_3Na$	98.2
Comparable Example 3	5.3×10^{-4}	$-SO_3H$	63.3

Examples 4 and 5 and Comparative Example 4

A polypropylene membrane having an average pore diameter of 0.4 μm, a porosity of 54%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-methoxyethyl acrylate gas (1.0 Torr) for 3 minutes, and glycidyl acrylate gas (0.7 Torr) for 7 minutes to promote surface graft polymerization. The resulting hydrophilic porous membrane having epoxy group introduced on its surface was sulfated in a 20% sodium sulfite solution (40°C) that had been acidified with sulfuric acid for 1, 12 and 24 hours to convert the epoxy group to sulfuric group.

Sample membranes of Examples 4 and 5 wherein the sulfate group on the porous membrane is in the form of Na salt were prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

Sample membrane of Comparative Example 4 wherein the sulfate group on the porous membrane is in the form of $-SO_3H$ was prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

The membranes were evaluated for its performance of HIV and gp120 removal by repeating the procedure of Example 1.

The results are shown in Table 3.

Table 3

	Sulfate group		% removal	
	Amount, mol/g	Type	HIV	gp120
Example 4	7.5×10^{-4}	$-SO_3Na$	99.5	$\geqq 95$
Example 5	6.4×10^{-4}	$-SO_3Na$	98.8	$\geqq 95$
Comparable Example 4	4.6×10^{-4}	$-SO_3H$	52.0	12

Comparative Examples 5 and 6

Various types of beads having sulfate group on their surface were compared for their performance of HIV removal. The beads were prepared to have capacity that would result in the amount of sulfate group nearly equivalent to the sulfated polypropylene membrane of Example 5 (see the total amount of SO_3Na relative mole ratio in Table 4). The sulfated beads were contacted with the HIV in 3 ml of 10% FCF-containing RPMI medium for 30 minutes by mixing with turning over the bottle. The performance of HIV removal was evaluated as in the case of Example 1.

The results are shown in Table 4.

Table 4

		Total amount of SO_3Na, relative mol ratio	% removal of HIV
Example 5	Sulfated propylene membrane	1.9×10^{-5}	99
Comparable Example 5	SP-Sepharose (manufactured by Pharmacia)	2.1×10^{-5}	18
Comparable Example 6	IR120B (manufactured by Organo)	1.9×10^{-5}	21

Comparative Examples 7 and 8

A polypropylene membrane having an average pore diameter of 0.4 μm, a porosity of 54%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-methoxyethyl acrylate gas (1.0 Torr) for 3 minutes, and methacrylic acid gas (0.8 Torr) for 5 minutes to promote surface graft polymerization and produce a hydrophilic porous membrane having carboxyl group introduced on its surface.

Sample membrane of Comparative Examples 7 wherein the carboxyl group on the porous membrane is in the form of Na salt was prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

Sample membrane of Comparative Examples 8 wherein the carboxyl group on the porous membrane is in the form of -COOH were prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

The amount of the carboxyl group on the membrane was determined by acid base titration to be 6.3×10^{-4} mol/g.

The sample membrane was set on Swinrock Filter Holder (manufactured by Nuclepore; diameter, 25 mm), and 5 ml of HIV-containing human plasma was filtered through the membrane to measure the performance of HIV removal by comparing the infectious titer before and after the filtration as in the case of Example 1.

The results are shown in Table 5.

Table 5

	Carboxyl group	% removal of	
	Content, mol/g	Type	HIV
Comparable Example 7	6.3×10^{-4}	-COONa	10
Comparable Example 8	6.3×10^{-4}	-COOH	10

The polycarboxylated membranes of both Na and H types exhibited the HIV removal of about 10%, which is significantly lower than the membrane of the present invention.

As described above, the material of the present invention is capable of removing the HIV and its related substances from blood and plasma. Therefore, the material of the invention may be used for adsorbing and removing the HIV and its related substances from the blood of the patient suffering from HIV infections (AIDS and ARC) in order to reduce the load of the patient and to thereby suppress further progress of the AIDS and improve the condition of the patient The material of the invention may also be used for reducing the HIV in blood and blood preparations to thereby reduce the risk of infection.

The material of the invention may be used in various conventional method and apparatus wherein a column or a membrane module is utilized. When the material of the pres nt invention is used in health-related or medical products for the purp se of preventing, treating or diagnosing the disease, the material of the invention may be us d alone or in combination with other materials by kneading, incorporating, r laminating the present material in other materials.

In addition to the use of the material of the present invention for the purpose of rem ving the HIV and its

related substances from a protein-containing solution such as plasma, the material of the invention may b als used for various products that may become in contact with the blood, body fluids, or their dropl ts under the conditions where the risk of HIV infection is involved. Ex mplary such products include medical and non-medical devices that are used for human and non-human organisms that are, or that may be infected by th HIV in medical fields and emergency treatments, as well as non-medical devices that are used by non-HIV-infected people for the purpose of preventing the HIV infection. The material of the present invention may also be used in products such as air filter for the purpose of providing an HIV-free environment.

Claims

1. A material for removing human immunodeficiency virus (HIV) and its related substances comprising a porous substrate having a plurality of pores, wherein
   said pores have sulfuric groups substantially in the form of a salt on their surface.

2. The material for removing HIV and its related substances according to claim 1, wherein
   said porous substrate is a porous membrane comprising a hydrophobic polymer having an average pore diameter of from 0.1 to 1 μm ; and
   said porous membrane has a polysulfuric compound loaded on its pore surface.

3. The material for removing HIV and its related substances according to claim 2, wherein said polysulfuric compound is loaded on the porous membrane via an intervening hydrophilic graft chain having a low protein adsorptivity.

4. The material for removing HIV and its related substances according to anyone of claims 1-3, wherein said sulfuric groups are in the form of their potassium or sodium salt.

5. The material for removing HIV and its related substances according to anyone of claims 2-4, wherein said polysulfuric compound is selected from the group consisting of dextran sulfate ; cellulose sulfate ; curdlan sulfate ; polymers and copolymers of sulfoethylacrylate, vinyl sulfate, and styrene sulfonic acid.

6. The material for removing HIV and its related substances according to anyone of claims 1-5, wherein said porous substrate comprises at least one member selected from the group consisting of polypropylene, polyethylene and polyvinylidene fluoride.

7. The material for removing HIV and its related substances according to anyone of claims 3-6, wherein said hydrophilic graft chain comprises at least one member selected from an acrylamide polymer, a polyether polymer, and a polyalkoxyalkylacrylate polymer.

8. The material for removing HIV and its related substances according to anyone of claims 3-6, wherein said intervening hydrophilic graft chain is a side chain grafted on said porous substrate, which comprises alkoxyalkyl acrylate and/or glycidyl (meth)acrylate.

9. The material for removing HIV and its related substances according to anyone of claim 2-8, wherein 1 x 10^{-4} mol/g or more of said sulfuric groups is loaded on the porous membrane substrate.

10. The material for removing HIV and its related substances according to anyone of claims 1-9, wherein said HIV related substance is at least one member selected from the group consisting of pathogenic glycoproteins constituting the HIV and their complexes with a biological component.

11. The material for removing HIV and its relates substances according to claim 10, wherein said pathogenic glycoprotein is at least one member selected from the group consisting of gp120 and gp160.

12. A method f r removing human immunodeficiency virus (HIV) and its related substances from blood by adsorption simultaneously with separation of plasma from the blood, wherein said method comprises the step of
    filtering the blood through a porous membrane having pores of an average pore diameter of from 0.1 t 1 μm, said pores having sulfuric groups substantially in the form of a salt on their surface

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 40 0972

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,A	EP-A-0 320 184 (KURARAY) * page 16; claims 1-10 *	1	B01J20/32 B01D15/00
A	EP-A-0 307 373 (ARES-SERONO) * page 2, line 58 - page 3, line 39 * * page 4, line 1-10 * * page 4, line 54-57 *	1,2,12	
A	EP-A-0 285 357 (UENO SEIYAKU) * page 2, line 25-29 * * page 3, line 56 - page 4, line 3 * * page 5, line 11-52 * * page 6, line 20-32 *	1,3-5	
A	EP-A-0 406 512 (MERRELL DOW) * page 2, line 35-43 *	11	
A	DATABASE WPI Week 9250 Derwent Publications Ltd., London, GB; AN 92-412038 & JP-A-04 309 502 (TANABE SEIYAKU) * abstract *	1	TECHNICAL FIELDS SEARCHED (Int.Cl.6) B01J B01D
A	US-A-5 071 880 (SUGO) * column 3-4; claims 1-8 *	6-8	
A	FR-A-2 669 535 (MEDGENIX)		
A	US-A-4 138 287 (ANDERSSON)		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 August 1995	Wendling, J-P

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(54) **Material for removing HIV and its related substances**

Material zur Entfernung von HIV und verwandten Substanzen

Matériau pour enlever le virus HIV et ses substances apparentées

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FIELD OF THE INVENTION

[0001] This invention relates to a material for removing human immunodeficiency virus (HIV) and the related substances from a body fluid. More illustratively, this invention relates to a membrane which is capable of selectively adsorbing the HIV and its related substances to enable removal of such substances from protein rich solutions such as blood, plasma, and solutions containing blood components.

BACKGROUND OF THE INVENTION

[0002] Many virus particles have an isoelectric point (pI) of from 3 to 6, and such viruses are negatively charged in neutral pH range. When such viruses are located in highly pure water, the viruses may be adsorbed onto an appropriate substrate by electrostatic interaction between the virus and the substrate.

[0003] Japanese Patent Application Laid-Open No. 3(1991)-123630 discloses a virus removing material comprising a substrate such as a porous membrane having polyvinylpyridinium structure thereon. This porous material, however, suffered from non-specific adsorption of proteins, and when the material was used for virus removal from fluids such as blood and plasma, which usually contain proteins at a high concentration, it failed to selectively adsorb the virus.

[0004] Another filter for removing virus from body fluids and other protein solutions is disclosed in Japanese Patent Application Laid-Open No. 2(1990)-167232. This filter comprises a regenerated cellulose membrane having a pore size smaller than the HIV particle, and the passage of the HIV particles upon filtration through the membrane is thereby inhibited. Such small pore size, however, also resulted in low filtration rate as well as frequent clogging. In addition, there has been reported that the pathogenicity of the HIV is found not only in the HIV particle, but also in the envelope proteins such as gp120 and gp160 (see, for example, *Microbiological Reviews*, Mar. 1993, pages 183-289, "Pathogenesis of HIV infection".) The pore size of this filter is not small enough to capture the gp120.

[0005] Another virus removing material is disclosed in WO89/01813, that utilizes biological affinity for the removal of the virus from the blood or plasma. This material comprises a substrate having immobilized thereon a virus-binding site (receptor) of a cell, that is found on the surface of the cell. Production of such material, however, involves complicated steps of isolation of the receptor from the cell and its purification followed by immobilization of the purified receptor onto the substrate. Use of such receptor of biological origin also resulted in an increased cost and some risk of unstable performance and alteration in the quality after storage.

[0006] Japanese Patent Application 2(1990)-36878 (USP 5,041,079) discloses a material for removing HIV and its related substances comprising a solid substance with a weakly acidic or weakly alkaline surface at a pH of from 2.5 to 6.9 or from 7.4 to 10.5. This material may typically have -COOH or -SO_3H on its surface, and there is clearly stated that "substances whose surface pH is in the range 7.0 to 7.3 (e.g. nonpolar polypropylene, quartz, and cation-exchange resins of -SO_3Na type) are unable to absorb HIV and/or its related compounds" (col. 2, lines 29 to 33 in USP 5,041,079). Furthermore, it has been confirmed that the body fluid would undergo an alteration in its pH upon contact with such an HIV removing material, and use of such material is not necessarily favorable for blood and other body fluids since such alteration in the pH may result in the denaturing of the proteins.

SUMMARY OF THE INVENTION

[0007] In view of the situation as described above, an object of the present invention is to provide a material by which HIV and its related substances can be removed from protein rich solutions such as blood and plasma preparations at a high selectivity and by a convenient operation.

[0008] To attain such object, there is provided by the present invention a material for removing HIV and its related substances comprising a porous substrate which is a porous membrane comprising a hydrophobic polymer having an average pore diameter of from 0.1 to 1 μm, wherein said pores have sulfuric group substantially in the form of a salt such as potassium or sodium salt loaded on its pore surface.

[0009] The porous membrane preferably comprises a hydrophobic polymer such as polypropylene, polyethylene or polyvinylidene fluoride.

[0010] The polysulfuric compound may be loaded on the porous membrane via an intervening hydrophilic graft chain having a low protein adsorptivity ; such as those comprising an acrylamide polymer, a polyether polymer, or a polyalkoxyalkylacrylate polymer such as polymethoxyethylacrylate.

[0011] Preferably, the intervening hydrophilic graft chain is a side chain grafted on the porous substrate, which comprises alkoxyalkyl acrylate and/or glycidyl (meth)acrylate. Alkyl and alkoxy are understood in the present application as meaning (C_1-C_4)alkyl and (C_1-C_4)alkoxy, respectively.

[0012] "Low protein adsorptivity" means that the affinity (adsorption) between the intervening hydrophilic graft chain

and various proteins in blood is low.

[0013] The polysulfuric compound may preferably be selected from dextran sulfate ; cellulos sulfate ; curdlan sulfate ; polymers and copolymers of sulfoethylacrylate, vinyl sulfate, and styrene sulfonic acid.

[0014] The material may preferably have 1 x 10^{-4} mol/g or more of the sulfuric group on the porous membrane substrate, preferably the sulfuric group is a salt of potassium or sodium.

DESCRIPTION OF THE INVENTION

[0015] In the present invention, the "material for removing human immunodeficiency virus (HIV) and its related substances" is a material that removes or inactivates the HIV and related substances, and preferably, a material that removes the HIV and its related substances from blood or other body fluids by adsorption onto the material.

[0016] The "HIV related substances" include glycoproteins constituting the HIV such as gp120 and gp160, that are reported to be pathogenic, and complexes of such glycoproteins with a biological component.

[0017] The material for removing HIV and its related substances comprises a porous substrate which is a porous membrane comprising a hydrophobic polymer having an average pore diameter of from 0.1 to 1 µm ; and said porous membrane has a polysulfuric compound in said porous membrane has a polysulfuric compound in the form of a salt loaded on its pore surface, namely, in the form of potassium salt ($-SO_3K$), sodium salt ($-SO_3Na$), or the like in contrast to the sulfuric group of proton type ($-SO_3H$) as employed in Japanese Patent Application Laid-Open No.2(1990)-36878 (USP 5,041,079). As mentioned above, when a protein solution such as a body fluid is brought in contact with the material having the sulfuric group of proton type on its surface, the protein that became in contact with the sulfuric group would undergo an alteration in its pH by ion exchange reaction, and the HIV inactivation thus proceeds simultaneously with the adsorption and denaturing of other proteins. In contrast to such material, the material wherein the sulfuric group is in the form of a salt exhibits reduced non-specific protein adsorption as well as improved removal of the HIV and related substances including the gp120.

[0018] The material for removing HIV and its related substances may preferably have 1 x 10^{-4} mol/g or more of the sulfuric group on its surface although the amount of the sulfuric group required may vary in accordance with the configuration of the material. When the amount of the sulfuric group is less than 1 x 10^{-4} mol/g, the material would exhibit an insufficient removal of the HIV and gp120. The material may more preferably have 2 x 10^{-4} mol/g or more of the sulfuric group preferably localized on its surface that becomes in contact with the liquid to be treated.

[0019] The characteristic feature of the material for removing HIV and its related substances of the present invention is its ability to selectively remove the HIV and its related substances from the liquid such as blood that contains a variety of proteins. In the treatment of AIDS, it is important to prevent the virus from spreading, to reduce the load imposed by the virus, and to inhibit the virus replication. Removal of HIV, gp120 and the like from the body fluid of the patient suffering from HIV blood conditions should result in the reduction of the virus load and prevention of the virus spread, and hence, in the improvement of the QOL (quality of life) of the patient and suppression of the disease progress.

[0020] The removal of the HIV and its related substances by the material of the present invention is represented by the reduction in the HIV infectious titer calculated in terms of median tissue culture infectious dose ($TCID_{50}$)/ml by the use of the material. For example, when a disk-shaped filter having a diameter of 25 mm is used by passing 2 ml of plasma having an infectious dose of from several tens to several hundreds $TCID_{50}$/ml (corresponding to the treatment of about 10 liters of plasma or about 20 liters or more of blood by a module having a membrane area of 0.5 m^2), the filter should exhibit an HIV removal of 85% or higher, and preferably, 90% or higher, and more preferably, 95% or higher.

[0021] In the present invention, loading of the sulfuric group on the material means chemical binding or immobilization of the sulfuric group on the material surface in a manner that the sulfuric group would not dissolve into water or blood. Such loading may be accomplished by various means, for example, by introducing a functional radical such as epoxy, amino, aldehyde, carboxyl, hydroxyl, or acid chloride group on the surface of the polymer material by such means as graft polymerization, coating, chemical modification, oxidization, and cross linking, and immobilizing the polysulfuric compound directly on the thus treated material by using or without using a coupling agent, or indirectly with an intervening spacer.

[0022] In a preferred method, a surface graft chain having a reactive functional radical may be introduced on the surface of the polymer material, and a polysulfuric compound may be immobilized on the reactive functional radical. In another method, the material surface is sulfated with sulfuric acid or sulfite to form a surface structure wherein a polysulfuric compound is loaded on the material surface. Preferably, such sulfation of the material surface is carried out after graft polymerizing a compound having epoxy group or hydroxyl group on the material surface.

[0023] The graft chain having a reactive functional radical may preferably comprise a monomer component such as glycidyl acrylate and glycidyl methacrylate having epoxy group therein, since the epoxy group may be changed to an amino group for reacting with a sulfuric group.

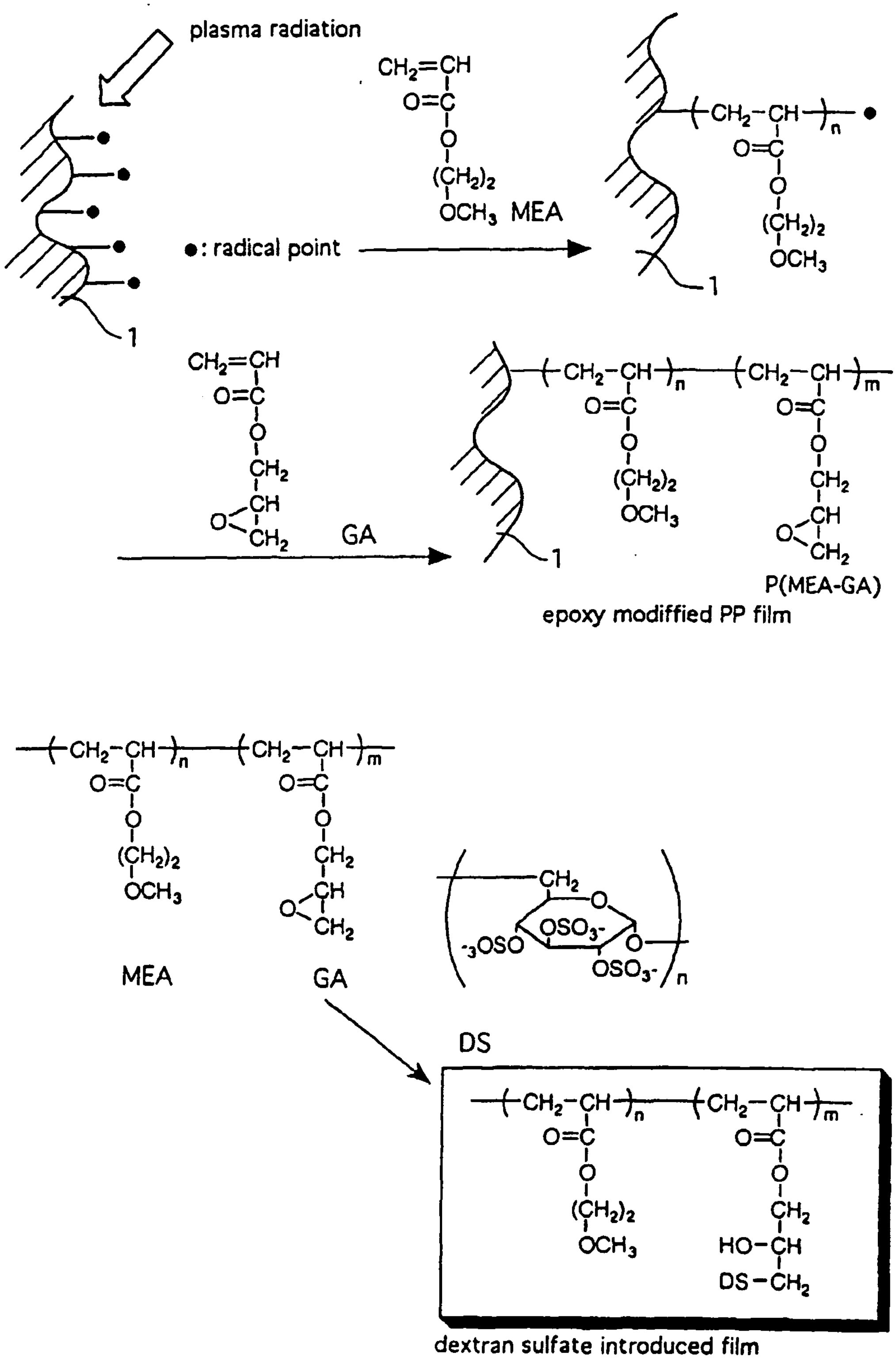
[0024] The polysulfuric compound is not limited to any particular type so long as it has a plurality of sulfuric groups in its molecule. Exemplary polysulfuric compounds are sulfated polysaccharides such as dextran sulfate, cellulose

sulfate, and curdlan sulfate ; and polymers and copolymers of a sulfonated monomer such as sulfoethylacrylate, vinyl sulfate, and styrene sulfonic acid. Preferably, the polysulfuric compound is immobilized on the material by forming a hydrophilic graft chain exhibiting a low protein adsorptivity which comprises a polymer having a glass transition temperature (Tg) of up to 300K such as an acrylamide polymer, a polyether polymer, a polyalkoxyalkylacrylate polymer, glycidyl (meth)acrylate or the like on the material surface ; introducing the above-described surface graft chain having a reactive functional radical ; and immobilizing the polysulfuric compound on the reactive functional radical.

[0025] An example of the methods for producing the material of this invention is shown schematically hereinafter.

[0026] The polyolefin membrane 1 such as polypropylene is irradiated with plasma to introduce radical points on the surface, and the surface is contacted with an alkoxy alkyl acrylate gas and/or a glycidyl (meth)acrylate gas to introduce graft polymerized side chains having active groups such as epoxy groups or hydroxyl groups.

[0027] Optionally, the active groups may be converted in other active groups, which may then be reacted with sulfuric groups, preferably in an aqueous solution.



[0028] The material for removing HIV and its related substances of the present invention is not limited in its configuration and may have any desired configuration such as a fiber, a filter, or a tube. Preferably, the material is a filter

having an average pore diameter of from 0.1 to 50 μm comprising a flat membrane, a hollow fiber, a woven fabric, or a nonwoven fabric.

[0029] When the material of the invention is used for filtering plasma or a culture medium, the filter may preferably hav an average pore diameter of 0.1 to 10 μm. The pore diameter of as small as less than 0.1 μm will result in an insufficient filtration rate. The average pore diameter as used herein is the value determined by Perm-Porometer (manufactured by Porous Materials, Inc.) described in ASTM-F316, and this value satisfactorily reflects the actual pore diameter.

[0030] Preferably, the filter may also have a water transmission of at least 10 ml/min/m²/mmHg, and more preferably, at least 100 ml/min/m²/mmHg when measured at a pressure of 0.7 kg/cm² and at 25°C ± 2°C, and then, the filter can be used at a relatively low filtration pressure.

[0031] When the material of the invention comprises a nonwoven fabric substrate, the nonwoven fabric substrate may comprise either a monofilament or a multifilament, but the filament should have an average diameter (an average of major diameter and minor diameter determined by observation with scanning electron microscope) of up to 100 μm and preferably, up to 50 μm. The membrane filter will then have a sufficient surface area, and hence, a sufficient adsorption site. It is also possible to use a profile filament, or alternatively, a porous filament. The filter material of the present invention may have a porosity (percentage of the pore volume) as determined by the following formula (A) of at least 20%, and preferably, at least 50%.

$$\text{Porosity (\%)} = \frac{\text{Volume of the pores}}{\text{Volume of (the pores and the filter material)}} \times 100$$

[0032] The material for removing HIV and its related substances of the present invention may comprise any desired material including natural polymers such as cellulose and its derivatives; and synthetic polymers such as polyolefin, polyamide, polyimide, polyurethane, polyester, polysulfone, and polyacrylonitrile. Preferably, the material of the invention may comprise a material with a high dimensional stability that may experience little swelling upon contact with water. Exemplary such materials include hydrophobic polymers such as polypropylene polyethylene and polyvinylidene fluoride. The material used in the present invention may preferably have its surface modified, polymerized or treated by hydrophylic materials to introduce sulfuric group and to prevent it from adsorption of proteins.

[0033] As described above, the solution treated by the material of the present invention may include various proteins in addition to the HIV and its related substance. Exemplary solutions that may be treated by the material of the invention include body fluids such as blood, plasma, serum and urine and a liquid containing such a body fluid; and tissue or cell culture medium, supernatant thereof, and a liquid containing such a culture medium or supernatant.

[0034] The blood or blood containing solution treated by the material of the present invention may preferably be treated while removing blood cells.

[0035] The solution treated that may possibly contain HIV or its related substance is contacted with the material of the present invention to thereby reduce the HIV activity or the risk of HIV infection. The material may be contacted with the solution by batchwise immersion of the material in the solution; or by continuous passage of the solution through the material in the form of a column or a filter, the filtration through the filter-shaped material being the most preferred.

[0036] When the material for removing the HIV and its related substance may be in the form of a porous filter or a bead, preferably the porous filter has an average pore diameter of from 0.1 to 1.0 μm, and has the sulfate group on its pore surface substantially in the form of a salt. It may be used as a horizontal filter in the filtration of blood to thereby accomplish plasma separation simultaneously with the removal by adsorption of the HIV and its related substances. Use of such filter also enables a convenient preparation of the HIV removing system.

[0037] The material and the method for removing HIV and its related substances of the present invention is further described by referring the Examples of the invention and Comparative Examples, which by no means limit the scope of the invention.

EXAMPLES

Examples 1 and 2 and Comparative Examples 1 and 2

[0038] A polypropylene (pp) membrane having an average pore diameter of 0.5 μm, a porosity of 58%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-methoxyethyl acrylate (MEA) gas (1.0 Torr) for 3 minutes, and glycidyl acrylate (GA) gas (0.7 Torr) for 7 minutes to promote surface graft polymerization. The resulting hydrophilic porous membrane having epoxy group introduced on its surface was immersed in aqueous ammonia of high concentration at 40°C for 48 hours to convert the epoxy group to amino group and produce an aminated hydrophilic porous membrane.

[0039] In the meanwhile, dextran sulfate (DS) (manufactured by Sigma; molecular weight, 5,000; degree of sulfation,

2.2) was oxidized with sodium periodate at a predetermined concentration (5% by weight) to produce aldehydated dextran sulfate.

[0040] The aminated hydrophilic porous membrane was reacted with the aldehydated dextran sulfate in water, and the resulting membrane was reduced with a 10 % by weight aqueous solution of $NaBH_4$ to strengthen the bond between the sulfate group and the membrane.

[0041] Sample membranes of Examples 1 and 2 wherein the sulfate group on the porous membrane is in the form of Na salt were prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

[0042] Sample membranes of Comparative Examples 1 and 2 wherein the sulfate group on the porous membrane is in the form of $-SO_3H$ were prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

[0043] The amount of the sulfate group introduced on the membrane was determined by acid base titration (NaOH-HCl back titration).

[0044] The sample membrane was set on a Swinrock Filter Holder (manufactured by Nuclepore; diameter, 25 mm), and 5 ml of HIV-containing human plasma (HIV infectious titer: 174 $TCID_{50}$/ml) was filtered through the membrane to measure HIV infectious titer and content of p24 before and after the filtration to thereby calculate the removal of the HIV and p24. The results are shown in Table 1. Removal of gp120, which is the glycoprotein found in the HIV envelope, was measured by filtering 5 ml of 2% FCS-containing RPMI 1640 medium containing HIV-containing human plasma through the membrane to measure the concentration of gp120 before and after the filtration, from which the gp120 removal was calculated. The results are also shown in Table 1.

[0045] The HIV-containing human plasma was prepared by cultivating HIV (HTLV-IIIB) persistently infected cell, Molt-4/HIV in 10% FCS-containing RPMI1640 and collecting the HIV solution by centrifugation, and adding human plasma to the HIV solution.

[0046] The HIV infectious titer was determined in terms of median tissue culture infectious dose ($TCID_{50}$)/ml by observing the cell morphology with naked eye and fluorescent antibody technique.

[0047] The p24 antigen was quantitated with RETRO-TEK p24 Antigen ELSA manufactured by Cellular Products, Inc.

Table 1

	Sulfate group		% removal		
	Amount, mol/g	Type	HIV	gp120	p24
Example 1	7.2×10^{-4}	$-SO_3Na$	99.2	≥ 95	≥ 98
Example 2	3.7×10^{-4}	$-SO_3Na$	97.2	≥ 95	≥ 98
Comparable Example 1	7.2×10^{-4}	$-SO_3H$	54.4	62	58
Comparable Example 2	3.7×10^{-4}	$-SO_3H$	58.2	55	52

Example 3 and Comparative Example 3

[0048] A polyvinylidene fluoride membrane having an average pore diameter of 0.4 μm, a porosity of 64%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with glycidyl acrylate gas (0.7 Torr) for 3 minutes to promote surface graft polymerization and produce a porous membrane having epoxy group introduced on its surface.

[0049] In the meanwhile, amino group was introduced into dextran sulfate (manufactured by Sigma; molecular weight, 5,000; degree of sulfation, 2.2) by means of 4-aminobutyldiethoxymethylsilane, and the aminated dextran sulfate was immobilized on the above-described porous membrane having epoxy group introduced thereon.

[0050] Sample membrane of Example 3 wherein the sulfate group is in the form of Na salt was prepared by neutralizing the membrane with sodium hydroxide and thoroughly rinsing the membrane with distilled water.

[0051] Sample membrane of Comparative Example 3 wherein the sulfate group is in the form of $-SO_3H$ were prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

[0052] The membranes were evaluated for their performance of HIV removal by repeating the procedure of Example 1.

[0053] The results are shown in Table 2.

Table 2

	Sulfate group		% removal of
	Amount, mol/g	Type	HIV
Example 3	5.3×10^{-4}	$-SO_3Na$	98.2
Comparable Example 3	5.3×10^{-4}	$-SO_3H$	63.3

Examples 4 and 5 and Comparative Example 4

[0054] A polypropylene membrane having an average pore diameter of 0.4 μm, a porosity of 54%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-methoxyethyl acrylate gas (1.0 Torr) for 3 minutes, and glycidyl acrylate gas (0.7 Torr) for 7 minutes to promote surface graft polymerization. The resulting hydrophilic porous membrane having epoxy group introduced on its surface was sulfated in a 20% sodium sulfite solution (40°C) that had been acidified with sulfuric acid for 1, 12 and 24 hours to convert the epoxy group to sulfuric group.

[0055] Sample membranes of Examples 4 and 5 wherein the sulfate group on the porous membrane is in the form of Na salt were prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

[0056] Sample membrane of Comparative Example 4 wherein the sulfate group on the porous membrane is in the form of $-SO_3H$ was prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

[0057] The membranes were evaluated for its performance of HIV and gp120 removal by repeating the procedure of Example 1.

[0058] The results are shown in Table 3.

Table 3

	Sulfate group		% removal	
	Amount, mol/g	Type	HIV	gp120
Example 4	7.5×10^{-4}	$-SO_3Na$	99.5	≥ 95
Example 5	6.4×10^{-4}	$-SO_3Na$	98.8	≥ 95
Comparable Example 4	4.6×10^{-4}	$-SO_3H$	52.0	12

Comparative Examples 5 and 6

[0059] Various types of beads having sulfate group on their surface were compared for their performance of HIV removal. The beads were prepared to have capacity that would result in the amount of sulfate group nearly equivalent to the sulfated polypropylene membrane of Example 5 (see the total amount of SO_3Na relative mole ratio in Table 4). The sulfated beads were contacted with the HIV in 3 ml of 10% FCF-containing RPMI medium for 30 minutes by mixing with turning over the bottle. The performance of HIV removal was evaluated as in the case of Example 1.

[0060] The results are shown in Table 4.

Table 4

		Total amount of SO_3Na, relative mol ratio	% removal of HIV
Example 5	Sulfated propylene membrane	1.9×10^{-5}	99
Comparable Example 5	SP-Sepharose (manufactured by Pharmacia)	2.1×10^{-5}	18
Comparable Example 6	IR120B (manufactured by Organo)	1.9×10^{-5}	21

Comparative Examples 7 and 8

[0061] A polypropylene membrane having an average pore diameter of 0.4 μm, a porosity of 54%, and a thickness of 80 μm was irradiated with argon plasma (100 W, 0.1 Torr, 15 sec.), and the membrane was contacted with 2-meth-

oxyethyl acrylate gas (1.0 Torr) for 3 minutes, and methacrylic acid gas (0.8 Torr) for 5 minutes to promote surface graft polymerization and produce a hydrophilic porous membrane having carboxyl group introduced on its surface.

[0062] Sample membrane of Comparative Examples 7 wherein the carboxyl group on the porous membrane is in the form of Na salt was prepared by neutralizing the membrane in sodium hydroxide and thoroughly rinsing the membrane with distilled water.

[0063] Sample membrane of Comparative Examples 8 wherein the carboxyl group on the porous membrane is in the form of -COOH were prepared by treating the membrane with 0.1N dilute hydrochloric acid and rinsing the membrane with distilled water.

[0064] The amount of the carboxyl group on the membrane was determined by acid base titration to be 6.3×10^{-4} mol/g.

[0065] The sample membrane was set on Swinrock Filter Holder (manufactured by Nuclepore; diameter, 25 mm), and 5 ml of HIV-containing human plasma was filtered through the membrane to measure the performance of HIV removal by comparing the infectious titer before and after the filtration as in the case of Example 1.

[0066] The results are shown in Table 5.

Table 5

	Carboxyl group	% removal of HIV	
	Content, mol/g	Type	
Comparable Example 7	6.3×10^{-4}	-COONa	10
Comparable Example 8	6.3×10^{-4}	-COOH	10

[0067] The polycarboxylated membranes of both Na and H types exhibited the HIV removal of about 10%, which is significantly lower than the membrane of the present invention.

[0068] As described above, the material of the present invention is capable of removing the HIV and its related substances from blood and plasma.

[0069] The material of the invention may be used in manufacturing various apparatuses, wherein a column or a membrane module is utilized. When the material of the present invention is used in manufacturing a device for the purpose of preventing, treating or diagnosing the disease, the material of the invention may be used alone or in combination with other materials by kneading, incorporating, or laminating the present material in other materials.

[0070] In addition to the above use of the material of the present invention the material of the invention may be also used for manufacturing various devices that may become in contact with the blood, body fluids, or their droplets under the conditions where the risk of HIV infection is involved. Exemplary such devices include medical and non-medical devices that are used for human and non-human organisms that are, or that may be infected by the HIV in medical fields and emergency treatments, as well as non-medical devices that are used by non-HIV-infected people for the purpose of preventing the HIV infection. The material of the present invention may also be used in manufacturing devices such as air filter for the purpose of providing an HIV-free environment.

Claims

1. A material for removing human immunodeficiency virus (HIV) and its related substances, characterized in that it comprises a porous substrate which is a porous membrane comprising a hydrophobic polymer having an average pore diameter of from 0.1 to 1 µm ; and said porous membrane has a polysulfuric compound in the form of a salt loaded on its pore surface.

2. The material according to claim 1, wherein said polysulfuric compound is loaded on the porous membrane via an intervening hydrophilic graft chain having a low protein adsorptivity.

3. The material according to claims 1 or 2, wherein the sulfuric groups of the polysulfuric compound are in the form of their potassium or sodium salt.

4. The material according to any one of claims 1 to 3, wherein said polysulfuric compound is selected from the group, consisting of dextran sulfate ; cellulose sulfate ; curdlan sulfate ; polymers and copolymers of sulfoethylacrylate, vinyl sulfate, and styrene sulfonic acid.

5. The material according to any one of claims 1 to 4, wherein said porous substrate comprises at least one member

selected from the group consisting of polypropylene, polyethylene and polyvinylidene fluoride.

6. The material according to any one of claims 2 to 5, wherein said hydrophilic graft chain comprises at least one member selected from an acrylamide polymer, a polyether polymer, and a polyalkoxyalkylacrylate polymer.

7. The material according to any one of claims 2 to 5, wherein said intervening hydrophilic graft chain is a side chain grafted on said porous substrate, which comprises alkoxyalkyl acrylate and/or glycidyl (meth)acrylate.

8. The material according to any one of claims 1 to 7, wherein 1 x 10-4 mol/g or more of said sulfuric groups is loaded on the porous membrane substrate.

9. The material according to any one of claims 1 to 8, in the configuration of a filter or a tube.

10. Use of a material as defined in any one of claims 1 to 9 for the manufacture of a medical or non-medical device for removing HIV and its related substances.

11. The use according to claim 10, wherein said material has the configuration of a fiber, a filter or a tube.

12. The use according to claims 10 or 11, wherein said HIV related substance is at least one member selected from the group consisting of pathogenic glycoprotein constituting the HIV and their complexes with a biological component.

13. The use according to claim 12, wherein said pathogenic glycoprotein is at least one member selected from the group consisting of gp120 and gp160.

Patentansprüche

1. Material zur Entfernung des menschlichen Immunschwächevirus (HIV) und seiner verwandten Substanzen, **dadurch gekennzeichnet, dass** es ein poröses Substrat umfasst, welches eine poröse Membran ist, die ein hydrophobes Polymer mit einem durchschnittlichen Porendurchmesser von 0,1 bis 1 um umfasst; und wobei die poröse Membran eine polyschwefelsaure Verbindung in Form eines Salzes aufweist, das auf ihrer Porenoberfläche aufgetragen ist.

2. Material nach Anspruch 1, wobei die polyschwefelsaure Verbindung auf der porösen Membran über eine vermittelnde, hydrophile Pfropfkette mit einem niedrigen Proteinadsorptionsvermögen aufgetragen ist.

3. Material nach den Ansprüchen 1 oder 2, wobei die schwefelsauren Gruppen der polyschwefelsauren Verbindung in Form ihrer Kalium- oder Natriumsalze vorliegen.

4. Material nach einem der Ansprüche 1 bis 3, wobei die polyschwefelsaure Verbindung aus der Gruppe ausgewählt ist, bestehend aus: Dextransulfat; Cellulosesulfat; Curdlansulfat (curdlan sulfate); Polymere und Copolymere von Sulfoethylacrylat, Vinylsulfat und Styrolsulfonsäure.

5. Material nach einem der Ansprüche 1 bis 4, wobei das poröse Substrat mindestens ein Element umfasst, das aus der Gruppe ausgewählt ist, die aus Polypropylen, Polyethylen und Polyvinylidenfluorid besteht.

6. Material nach einem der Ansprüche 2 bis 5, wobei die hydrophile Pfropfkette mindestens ein Element umfasst, das aus einem Acrylamidpolymer, einem Polyetherpolymer und einem Polyalkoxyalkylacrylatpolymer ausgewählt ist.

7. Material nach einem der Ansprüche 2 bis 5, wobei die vermittelnde, hydrophile Pfropfkette eine Seitenkette ist, die auf das poröse Substrat gepfropft ist, welches Alkoxyalkylacrylat und/oder Glycidyl(meth)acrylat umfasst.

8. Material nach einem der Ansprüche 1 bis 7, wobei 1 x 10^{-4} mol/g oder mehr der schwefelsauren Gruppen auf dem porösen Membransubstrat aufgetragen sind.

9. Material nach einem der Ansprüche 1 bis 8 in der Anordnung eines Filters oder einer Röhre.

10. Verwendung des Materials, wie es in einem der Ansprüche 1 bis 9 definiert ist, für die Herstellung einer medizinischen oder nicht-medizinischen Vorrichtung zur Entfernung von HIV und seiner verwandten Substanzen.

11. Verwendung gemäß Anspruch 10, wobei das Material die Anordnung einer Faser, eines Filters oder einer Röhre besitzt.

12. Verwendung gemäß den Ansprüchen 10 oder 11, wobei die HIV verwandte Substanz mindestens ein Element ist, das aus der Gruppe ausgewählt ist, die aus einem pathogenen, das HIV aufbauenden Glycoprotein und dessen Komplexen mit einer biologischen Komponente besteht.

13. Verwendung gemäß Anspruch 12, wobei das pathogene Glycoprotein mindestens ein Element ist, das aus der Gruppe, die aus gp120 und gp160 besteht, ausgewählt ist.

Revendications

1. Matériau pour retirer le virus de l'immunodéficience humaine (VIH) et les substances apparentées, caractérisé en ce qu'il comprend un substrat poreux qui est une membrane poreuse comprenant un polymère hydrophobe ayant un diamètre moyen de pores de 0,1 à 1μm, et ladite membrane poreuse a un composé polysulfurique sous forme d'un sel chargé sur sa surface poreuse.

2. Matériau selon la revendication 1, dans lequel ledit composé polysulfurique est chargé sur la membrane poreuse par le biais d'une chaîne de greffage hydrophile intermédiaire ayant un faible pouvoir adsorbant à l'égard des protéines.

3. Procédé selon la revendication 1 ou 2, dans lequel les groupes sulfuriques du composé polysulfurique sont sous forme de leur sel de potassium ou de sodium.

4. Matériau selon l'une quelconque des revendications 1 à 3, dans lequel ledit composé polysulfurique est choisi dans le groupe consistant en le sulfate de dextrane, le sulfate de cellulose, le sulfate de curdlane, les polymères et copolymères d'acrylate de sulfoéthyle, de sulfate de vinyle et d'acide styrènesulfonique.

5. Matériau selon l'une quelconque des revendications 1 à 4, dans lequel ledit substrat poreux comprend au moins un élément choisi dans le groupe consistant en le polypropylène, le polyéthylène et le poly(fluorure de vinylidène).

6. Matériau selon l'une quelconque des revendications 2 à 5, dans lequel ladite chaîne de greffage hydrophile comprend au moins un élément choisi parmi un polymère d'acrylamide, un polymère polyéther et un polymère poly (acrylate d'alcoxyalkyle).

7. Matériau selon l'une quelconque des revendications 2 à 5, dans lequel ladite chaîne de greffage hydrophile intermédiaire est une chaîne latérale greffée sur ledit substrat poreux, qui comprend de l'acrylate d'alcoxyalkyle et/ou du (méth)acrylate de glycidyle.

8. Matériau selon l'une quelconque des revendications 1 à 7 dans lequel 1×10^{-4} mol/g ou plus desdits groupes sulfuriques est chargé sur le substrat constitué par la membrane poreuse.

9. Matériau selon l'une quelconque des revendications 1 à 8, dans la configuration d'un filtre ou d'un tube.

10. Utilisation d'un matériau selon l'une quelconque des revendications 1 à 9 pour la fabrication d'un dispositif médical ou non médical pour retirer VIH et les substances apparentées.

11. Utilisation selon la revendication 10, dans laquelle ledit matériau a la configuration d'une fibre, d'un filtre ou d'un tube.

12. Utilisation selon la revendication 10 ou 11, dans laquelle ladite substance apparentée à VIH est au moins un élément choisi dans le groupe consistant en les glycoprotéines pathogènes constituant le VIH et leurs complexes avec un composant biologique.

13. Utilisation selon la revendication 12, dans laquelle ladite glycoprotéine pathogène est au moins un élément choisi dans le groupe consistant en gp120 et gp160.